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Interacting impacts of damming and metal pollution on the Pyrenean Brook Newt, *Calotriton asper* (Dugès, 1852)

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Abstract. The Pyrenean Brook Newt, *Calotriton asper*, is a key component of the ecosystem in Pyrenean headwater streams. We here describe the effects of metal pollution and damming on a newly discovered population of *C. asper* by assessing differences in population density and body size of subpopulations above and below the dam, using available environmental data to discern their causes. We found a decrease in population density and body size in the upstream subpopulation due to metal pollution. Water diversion by the dam reduced the metal pollution impact downstream. Water flow reduction and warming due to damming are threats to this *C. asper* population, and the issue is particularly pressing in the Pyrenees due to the expected reduced water availability caused by climate change. We also tested for chytridiomycosis in this population but did not find any infected individuals despite their proximity to an infected population.

Keywords. Caudata; Salamandridae; conservation ecology; ecotoxicology; chytridiomycosis

Introduction

Freshwater ecosystems are biodiversity hotspots that contain more than one third of all vertebrate species (Dudgeon et al., 2006; Bailan et al., 2008). One of the most threatened groups in these ecosystems are amphibians, with at least 40% of their species experiencing worldwide population declines (Bishop et al., 2012; Colomer et al., 2014). Amphibians depend on water quality and availability for their survival and reproduction, which makes them highly sensitive to threats like climate change, metal pollution, stream regulation, and water-associated diseases, among others (Bednarek, 2001; Lessard and Hayes, 2003; Wake, 2007; Walls et al., 2013; Dovick et al., 2020).

These threats are especially relevant to mountain headwater streams, one of the most unique and vulnerable types of freshwater ecosystems. These environments are characterized by particular hydrological and morphological conditions, cold and oligosaline waters, and conspicuous seasonal variations in flow (Milner and Petts, 1994; Giller and Malmqvist, 1998; Maddock, 1999; Freeman et al., 2007). The morphology of these streams causes their waters to have high energy, and this feature has historically been taken advantage of with the construction of hydroelectric dams that impair the connectivity of streams and reduce their natural water flow, affecting their physical and chemical characteristics (Mor et al., 2018; Zarfl et al., 2019). In recent decades, this type of hydromorphological alteration has been coupled with an increase in metal and metalloid concentrations in water and sediments from the proliferation of industries and mining, which may affect many kinds of freshwater organisms due to their bioaccumulation in the trophic chain (Colas et al., 2013; Gessner and Tlili, 2016).

Field evidence of amphibian population declines caused by metal and metalloid pollution is lacking (Chen et al., 2009; Gardner et al., 2018) but, in the current climate change context, the impact of metal pollution on headwater streams could become exacerbated. A good region for studying potential amphibian population declines are the Pyrenees Mountains, a range in the Mediterranean Basin that separates the Iberian Peninsula from the rest of Europe. Climate models predict a decrease in precipitation of 10.7–14.8% and a mean increase in temperature between 2.8–4.0°C in these mountains by the end of the 21st century, accompanied by a decrease of the maximum accumulated snow level on which streams depend for maintaining a natural and sufficient water flow (López-Moreno et al., 2008, 2009; Cramer et al., 2018). As a result, water warming and flow reduction caused by climate change in headwater streams could exacerbate existing threats of metal

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pollution and damming, by directly threatening the water availability and quality necessary for healthy amphibian communities. This situation highlights the need for analyses of the potential impacts of these threats on vulnerable amphibian populations in this region.

An emblematic and protected amphibian species found in Pyrenean headwater streams is *Calotriton asper*. This endemic newt is found throughout the Pyrenees in Spain, France, and Andorra, reaching the pre-Pyrenees in some locations (Fig. 1). It is a rheophile species, adapted to a benthic life spent predominantly in cold and fast-flowing mountain streams with steep slopes, scarce vegetation, and benthos dominated by stones and gravel. These newts prefer areas with enough shelter to hide from predators, such as under rocks and between vegetal debris (Montori et al., 2008; Montori and Llorente, 2014).

Calotriton asper plays an important role in the energy flow pathways of headwater stream ecosystems where they function as prey, competitors, and predators in aquatic food webs. They prey on macroinvertebrates and drive top-down trophic cascades (Sánchez-Hernández, 2020). Optimally, their diet consists of stonefly, mayfly, caddisfly, and true fly larvae. They can also act as generalists if needed and are able to consume other amphibians, such as larvae and metamorphs of *Salamandra salamandra* (Linnaeus, 1758), which makes it rare to find these two species in microsympatry (Montori, 1988; Sánchez-Hernández, 2020). At the same time, these newts represent an important component of the energy budget for higher trophic levels, such as fish, snakes, and mammals.

The River Trout, *Salmo trutta*, is the main predator of *C. asper*, but any medium-sized fish is a potential predator (Montori, 1988). Predatory fish are a major force that structures amphibian assemblages as they can extirpate local populations, altering their distribution and abundance patterns (Vredenburg, 2004). On the southern slopes of the Catalan Pyrenees, newts and trouts are found predominantly in allopatry, but some populations are sympatric with only spatial and trophic segregation (Montori, 1988; Montori et al., 2006). Moreover, the main dietary components of trout are shared by newts (Sánchez-Hernández et al., 2019), which may cause competition between these two species for basic resources. All these facts make *C. asper* a key species for assessing energy and nutrient flow pathways in Pyrenean headwater streams, and declines in its populations may drive negative consequences for the structure and function of these ecosystems (Davic and Welsh, 2004; Preston and Johnson, 2012; Sánchez-Hernández, 2020).

Population data for *C. asper* are scarce, and there is only one pre-Pyrenean population that has been described in detail, with an estimated density of 3500–5500 individuals in a 1.5-km long stream (Montori, 1988; Montori and Llorente, 2014). In the centre of its range this species should be frequent and abundant (Montori and Llorente, 2014) but in marginal areas it comprises fragmented and less abundant populations, complicating the comparison between population densities found in the scientific literature. For example, densities in areas of Basque Country in northern Spain, near the western limit of the species' range, are estimated at only 17–72 individuals per kilometre of stream length (Gosá and Bergerandi, 1994; Arrayago et al., 2005).

The major threats to *C. asper* are the loss and degradation of aquatic habitats, hydrological alteration through dams and weirs, infrastructure development of skiing stations, diseases, pesticide and metal pollution, salmonid introductions, and climate change (Montori et al., 2002; Daszak et al., 2003; Colomer et al., 2014; IUCN SSC Amphibian Specialist Group, 2022). The species is listed as Least Concern (LC) in the *IUCN Red List* but it is also included under the category of Special Interest in the *National Catalogue of Endangered Species* (IUCN SSC Amphibian Specialist Group, 2022). The Spanish population of *C. asper* by itself is also considered Near Threatened (Montori et al., 2002), is protected by Spanish Royal Decrees of 1980 and 1986, and is included in the *Convention on the Conservation of European Wildlife and Natural Habitats* and in Annexes II and IV of the *EU Habitats Directive*. In this regard, new research is required to determine the current population sizes, distributions, and trends of this species, and to analyse threats and actions needed to improve its conservation (IUCN SSC Amphibian Specialist Group, 2022).

Despite the sensitivity of amphibians to hydrological alterations and considering that the hydrological regime plays a significant role in the structuring of amphibian communities, the impact of stream regulation on amphibians has rarely been assessed (Wassens and Maher, 2011). Damming threatens stream ecosystems by causing habitat degradation and fragmentation, flow reduction, changes in sediment transport, water temperature increase, deterioration in water quality, biodiversity loss and spreading of water-associated diseases (Bednarek, 2001; Lessard and Hayes, 2003).

Another important threat to amphibians, including *C. asper*, is chytridiomycosis, an emerging wildlife disease caused by the pathogenic fungi *Batrachochytrium dendrobatidis* (Bd) and *B. salamandrivorans* (Bsal), which have caused significant declines of amphibian populations around the world (Wake, 2012; Van Rooij et al., 2015), including across Spain, with cases on *C. asper* reported in Catalonia and the Pyrenees (Martínez-Silvestre et al., 2020; Romero-Zambrano et al., 2021). As a stream breeder, *C. asper* has relatively low fecundity, and as a habitat specialist in headwater streams, it is susceptible to these fungi, which grow faster in cool temperatures, have a wide host range, achieve high virulence, and produce asymptomatic infection in larvae. Moreover, chytrid fungi may survive outside the host (Van Rooij et al., 2015). Awareness of infectious diseases that threaten wildlife, especially amphibians, has increased in recent years but they generally remain poorly studied and understood (Duffus and Cunningham, 2010). Field monitoring of wild populations, including asymptomatic individuals, coupled with management practices, must be performed, and improved in order to understand the distribution of this disease and prevent its further spread (Athan et al., 2005; Martínez-Silvestre et al., 2020; Romero-Zambrano et al., 2021).

Our study focuses on a small Pyrenean headwater stream affected by a dam and by metal and metalloid pollution. The main objective was to produce a case study to characterise the population of *C. asper* in this stream and understand how damming, metal pollution, and the potential prevalence of chytridiomycosis affect it. We hypothesize that the differences in the metal and metalloid concentrations of the water above and below the dam will have a negative impact on the density and body size of *C. asper*.

Materials and Methods

Study Area. We conducted our study in a stream locally known as Ribera del Catllar (ca. 42.3637°N, 2.2753°E,

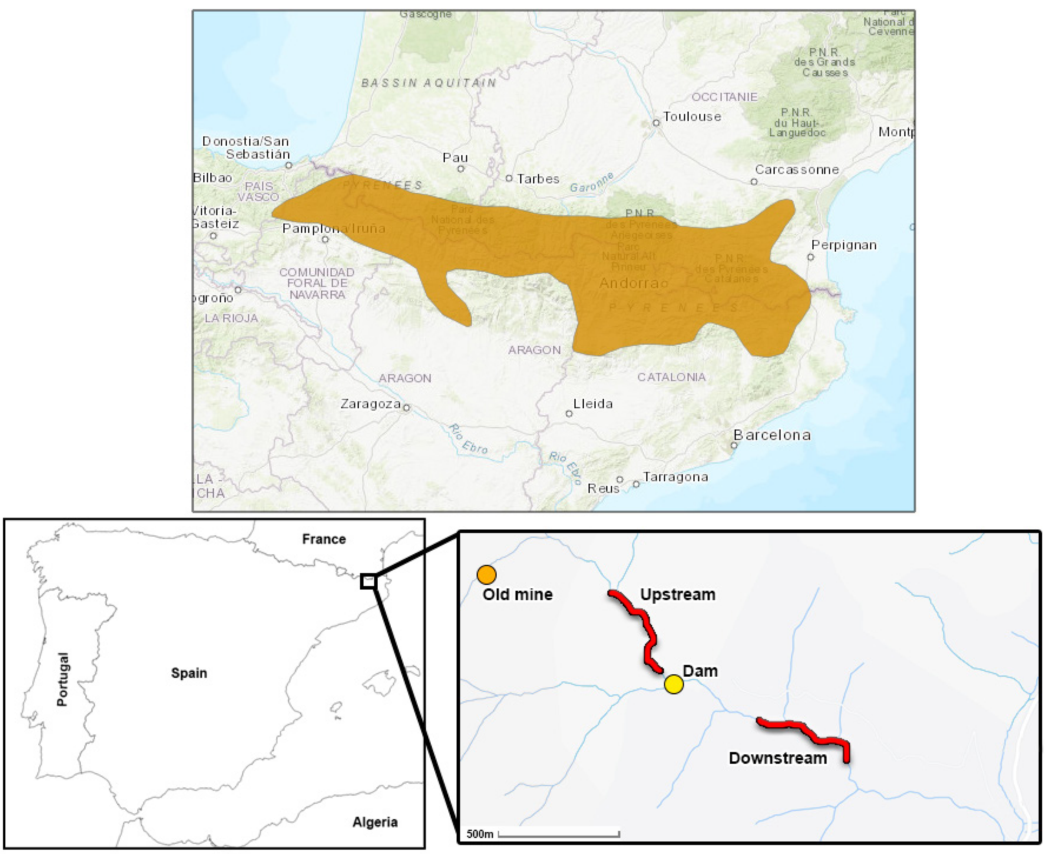

Figure 1. The main map shows the estimated current range of *Calotriton asper* in the Pyrenees Mountains along the border between France (to the north) and Spain. Andorra is in the centre east portion of the range. The map at lower left shows the position of the Catllar Stream basin in northern Spain with its main tributaries. The map at lower right shows the two survey transects, identified by red lines (Upstream and Downstream), the dam (yellow circle), and the abandoned mine (orange circle). Catllar Stream is a tributary of the Ter River in Catalonia.

elevation 1200–1600 m), a tributary of the Riu Ter [Ter River] in Catalonia, Spain. A hydroelectric dam diverts most of the upstream water to a hydroelectric facility and channels water from the adjacent western stream to the downstream section through a metal platform (López-de Sancha et al., 2022). We selected two transects of 500 m length above and below of the dam for our surveys. An old, abandoned antimony mine that leaks metals and metalloids into the environment is present west of the highest part of the upstream transect (Fig. 1). Physical and chemical characteristics of the stream were assessed in a previous study (López-de Sancha., 2022; López-de Sancha et al., 2022) and are reviewed in the Results section below.

Habitat characterization and newt surveys. Before we began our surveys, we assessed transects by identifying all potential newt habitats following the methodology proposed by Montori et al. (2008). Newt surveys were performed during July and August 2021, with searches of each transect from approximately 21:00–03:00 h when newts are most active (Hervant et al., 2000). The same search effort was maintained for each transect. We surveyed transects counter-current in order to avoid generating water turbidity and conducted active visual searches for newts in the riverbed and in typical *C. asper* habitats along the stream's edges using a small hand net. Two surveys of each transect were conducted on consecutive nights in order to estimate subpopulation density. We used a two-event mark-recapture methodology, with the first survey considered a capture and the second a recapture, allowing us to estimate newt numbers from the number of recaptures (Chapman, 1951). Because of the initially low number of individuals encountered in the stream, we doubled our sampling effort, performing two capture and recapture surveys for each transect. Individuals from both samples for the capture and recapture survey type were grouped and counted once. Individuals that were found in both events for a survey type were not considered for the calculations to avoid counting an individual twice.

We measured snout-to-vent length (SVL) and weighed and sexed each individual (Trochet et al., 2019). Newts were returned to the location of capture. A photograph of the venter was taken to allow individual identification to confirm recaptures because these newts have ventral colour patterns that differ between individuals. If *Salamandra salamandra* larvae were found during the newt surveys, they were also counted in order to assess the co-occurrence of both species and its possible implications.

Fish community sampling. Prior to newt surveys**,** we used electrofishing gear (Electracatch WFC4 High Voltage Pulsed DC Electrofishing System, Electracatch International, Wolverhampton, United Kingdom) to sample the fish community above and below the dam in July, in order to assess the predation pressure on this *C. asper* population. The electrofishing equipment was set at 225 V with a Direct Current (DC) duty-cycle and we followed the IBICAT methodology (ACA, 2010) to meet European norms (CEN, 2003). Electrofishing targeting fish has been shown to have no detrimental effects on amphibians (Gilbert et al., 2017; Morrison, 2019; Carrera-Suárez and Catchpole, 2021). A 100-m-long transect, that included the entire width of the stream, was delimited using fishing nets. We then performed three consecutive catches removing all captured fish to avoid recaptures. Fish were weighed and their standard length (tip of the snout to the posterior end of the last vertebra) was measured.

Chytridiomycosis detection. Captured newts were swabbed 15 times in the abdominal area and 15 more times divided between the fingers of all four legs. Cotton swabs were frozen at –20°C until analysis for the detection of Bd and Bsal could be performed (PCR protocol from Blooi et al. 2013).

Ethics and biosecurity. Capture permits for *C. asper* and trout were granted by the Catalan government and biosecurity measures were in place throughout our surveys to avoid contamination of the stream, by using a new pair of gloves to handle each newt and cleaning both boots and hand nets after each survey using a 5% bleach solution to avoid spreading *Bd* and *Bsal* spores.

Statistical analysis. Statistical analyses were performed using RStudio software v1.2.5033 (RStudio Team, 2019). Newt population density estimates above and below the dam and the standard error of the estimator were obtained by using the *nChapman* and the *seChapman* functions in the R package *recapr,* v0.4.3 (Tyers, 2020). Moreover, the *ciChapman* function was used to obtain 95% confidence intervals of estimated population density by bootstrapping and using a normal distribution (Montori et al., 2008). Even though the *ciChapman* function is not a statistical measure to test for population differences, when considering estimated population densities above and below the dam a lack of overlap in confidence intervals would suggest that significant differences exist between the sampled subpopulations (Williams et al., 2002).

Differences for weight and length measurements in subpopulations of *C. asper* above and below the dam were verified using a *t*-test. Data were checked for normality and homoscedasticity assumptions using a Shapiro-Wilk and a Levene test respectively.

In order to assess the possible differences in the physiological condition (relationship between weight and length) between subpopulations, an analysis of covariance (ANCOVA) was conducted using the weight (response variable) and length (covariate) values of each subpopulation on both sides of the dam (categorical factor). The assumption of homogeneity of slopes in the linear regression of the length and weight variables was tested by analysing the interactions between each covariate, using the location from the dam as a factor (García-Berthou and Moreno-Amich, 1993; Merciai et al., 2017; Latorre et al., 2019). The proportion of individuals found with deformities above (3 of 28) and below (9 of 59) the dam was compared with a two-proportions Z-test, using the *prop.test* function in RStudio.

The fish population density was estimated using the Carle-Strub method in the *Simple Fisheries Stock Assessment Methods* R package (Ogle et al., 2019), based on the maximum likelihood estimation of population size from removal data (Carle and Strub, 1978). Length and weight classes of captured fish were established based on standard lengths and body weight. We established seven length classes with an initial class including individuals measuring 0–2.9 cm, five classes defined by intervals of 2.4 cm each, and one class of individuals longer than 15.5 cm. Eight weight classes were established with the initial one including individuals weighing from 0–0.9 g, six intervals of 7.4 g each, and one class of individuals weighing > 46 g. A Chi-Square test was performed with using observed frequencies of fish in each length and weight class in order to compare the observed proportions to the expected ones if there were no differences between both trout subpopulations above and below the dam.

Results

Prior data. Information about water characteristics, metal and metalloid concentration, and food resource (macroinvertebrate) availability of Ribera del Catllar was obtained from previous studies and their data interpretations (López-de Sancha, 2022; López-de Sancha et al., 2022). These showed that there was reduced water flow below the dam with higher conductivity and pH. This downstream water was slightly warmer than upstream water, but this difference was just outside the range for being statistically significant (upstream water temperature 5.2 ± 1.6°C, downstream water temperature 6.6 ± 1.6°C; $p = 0.06$). The biomass of stonefly, mayfly, caddisfly, and true fly larvae (food resources preferred by *C. asper*) did not differ above and below the dam. The concentration of arsenic in the water, and of nickel, copper, and arsenic in biofilms, was higher above the dam than downstream.

Newt population density. A higher population density of *C. asper* was observed below the dam. There was no overlap between the respective upstream and downstream confidence interval estimates (Table 1).

Newt body size. Body size analysis showed that the subpopulation of *C. asper* below the dam had heavier ($t = 4.35$, $p < 0.001$) and longer ($t = 4.93$, $p < 0.001$) bodies than the subpopulation above the dam. The ANCOVA analysis showed that both subpopulations had a similar physical condition overall: a significant linear relationship between length and weight was observed, but the relative position from the dam had no effect on the association between weight and length of the newts (Table 2).

Habitat characterization. The predominant habitat type found in Catllar Stream were places providing natural cover (big rocks, caves, roots, bushes, and logs), pools, and waterfalls (Table 1), indicating that the stream has abundant and suitable habitats for *C. asper* above and below the dam.

Predation and competition. *Salmo trutta* was the only fish species found during our survey. Differences in the estimated fish population densities above and below the dam were not statistically significant. The estimated total biomass was slightly higher downstream but, despite this, once classified into weight and length classes (Table 3), no significant differences in fish size between subpopulations was found (weight classes: $\chi^2 = 6.948$, $p = 0.435$; length classes: $\chi^2 = 3.999$, $p = 0.677$). This indicates that subpopulations of *C. asper* above and below the dam were under a similar predation pressure from trout. In regard to competition, *Salamandra salamandra* larvae were predominantly found in the upstream transect ($n = 27$) with only three larvae found downstream, suggesting that salamander larvae may experience less competition at the reduced population density of *C.asper* above the dam.

Deformities. Individuals with limb deformities (Fig. 2) were encountered in both transects, with a higher proportion upstream (15.3%) than downstream (10.7%). These differences were not statistically significant ($\chi^2 = 0.058$, $p = 0.809$).

Chytridiomycosis. Results of the PCR analysis indicated that there was no presence of *Bd* or *Bsal* in any *C. asper* individual sampled in Catllar Stream.

Table 1. Habitat and population parameters for *Calotriton asper* in Catllar Stream, Vilallonga del Ter Municipality, Catalonia Autonomous Region, Spain, grouped by relative position to the dam. Habitat parameters include the numbers of pools, waterfalls and natural cover, added to produce the total. Results include the numbers of individuals captured during two sampling events, the number of newts recaptured, and the estimated population density (with standard error), as well as body size characteristics for each subpopulation. Numbers printed in bold are values for which the upstream and downstream subpopulations differed significantly from each other. Confidence intervals for the population density estimates are present for normal and bootstrap estimations.

Parameters	Upstream	Downstream
Habitat		
Pools	97	77
Waterfalls	73	58
Natural cover	230	293
Total habitat	400	428
Average stream width (m)	3.59 ± 0.58	4.20 ± 1.06
Average water depth (m)	0.31 ± 0.05	0.33 ± 0.06
Results		
1st sampling	16	32
2nd sampling	18	32
Recaptures	6	5
Estimated density	45.1 ± 9.9	180.5 ± 56.1
95% C.I. (normal)	25.7–64.6	70.5–290.5
95% C.I. (bootstrap)	28.4–106.7	107.9–543.5
Average weight (g)	10.7 ± 0.5	**13.2 ± 0.3**
Average. TL (mm)	127.8 ± 1.7	**137.3 ± 1.6**
Average SVL (mm)	70.4 ± 1.0	**77.1 ± 0.8**

Table 2. ANCOVA results of linear regression analyses to assess slope homogeneity and condition of *Calotriton asper* from Catllar Stream, Vilallonga del Ter Municipality, Catalonia Autonomous Region, Spain. Homogeneity was assessed snout–vent length (SVL) in relation to weight, with the interactions of these variables also included. Assessments were conducted using the relative position above and below the dam as the factor. Degrees of freedom = 1 for all the results.

Function	Sum.Square	*F*	*p*
Homogeneity Assessment			
SVL	246.11	101.07	< 0.001
Position (SVL)	2.57	1.05	0.30
SVL*Position	1.89	0.78	0.38
TL	268.09	112.69	< 0.001
Position (TL)	0.18	0.08	0.78
TL*Position	0.26	0.11	0.74
Newt condition			
SVL	340.09	140.05	< 0.001
Position (SVL)	8.12	3.34	0.07
TL	346.36	147.15	< 0.001
Position (TL)	0.92	0.39	0.54

Discussion

In order to conserve amphibian populations, we need to characterize them, assess their threats, and project future plans for their management. As a Pyrenean endemic, *C. asper* has a small range and management of small headwater streams could be decisive for the survival of these populations (Montori et al., 2007). Because habitat alteration and degradation are potentially reversible, defining the critical factors needed for restoring and conserving high-quality habitats for amphibians is essential if we aim to maintain the ecosystem structure in headwater streams (Lowe and Bolger, 2002; Semlitsch, 2002; Stoddard and Hayes, 2005).

The population of *Calotriton asper* we studied is the first record of this species, for the Catllar Stream and Vilallonga del Ter Municipality, but its presence had previously been recorded in the same 10 x 10 km distribution grid where the stream is found (Villares and Ruiz, 2020). The population is located on the southern slopes of the range of the species, and it is found in a habitat type with suitable conditions (Montori et al., 2008; Montori and Llorente, 2014). If abiotic conditions are favourable and food resources are abundant, the main driver of population density for *C. asper* is the availability of suitable habitat and natural cover (Montori et al., 2008). As seen in the habitat characterization of this study and in the previous environmental characterization, Catllar Stream presents favourable conditions for the species both above and below the dam: a headwater stream with steep slope and low water temperature, abundant and variable habitats and natural cover, low vegetation along the stream margins, and benthos dominated by rocks and gravel (Montori and Llorente, 2014). Therefore, a high population density should be expected. Instead, the population density was found to be similar to those at the edges of the species' range (Gosá and Bergerandi, 1994; Arrayago et al., 2005).

Density differences were also observed between subpopulations above and below the dam, with a higher density downstream. It is known that *C. asper* is a sedentary species with constant distribution and with annual movements up and down a stream of < 50 m, and thus their population density remains fairly constant throughout the year (Montori et al., 2008, 2012). Moreover, newts from the upstream subpopulation were smaller, even though the physiological condition of both subpopulations did not differ. Consequently, the newt population in Catllar Stream might be affected by adverse biotic or abiotic conditions causing population density and body size differences between subpopulations above and below the dam.

Table 3. Data for trout (*Salmo trutta*) captured in Catllar Stream, Vilallonga del Ter Municipality, Catalonia Autonomous Region, Spain. Shown are the number of captured individuals (*n*), the estimated population density per 100 linear metres of the stream ($Density_{100}$), means ± standard errors for standard length (SL) and weight (W), estimated density per hectare ($Density_{ha}$), estimated biomass per hectare (BM), and average transect width (ATW).

Position	*n*	$Density_{100}$	SL (cm)	W (g)	$Density_{ha}$	BM (kg/ha)	ATW (m)
Upstream	30	33 ± 3	9.3 ± 4.6	22.1 ± 26.7	866 ± 70	0.57	3.65
Downstream	24	32 ± 5	9.0 ± 3.7	17.7 ± 14.3	877 ± 126	0.73	3.81

Interactions of newts with salamanders and trout. Biotic interactions, such as interspecific competition and predation, could be causing differences between subpopulations above and below the dam, but this does not seem to be the case. While *S. salamandra* and *C. asper* are trophic competitors and coexist geographically, they were rarely found in microsympatry, due to predation by *C. asper* larvae on *S. salamandra* larvae and the dominance of *C. asper* when competing with these larvae for trophic resources (Montori, 1988). In our study, only three larval *S. salamandra* were found downstream from the dam where the density of *C. asper* was higher, while they were more frequently encountered upstream where the density of *C. asper* is lower. These results confirm those from other studies (Arrayago et al., 2005; Guillaume, 2006) and indicate the displacement of *S. salamandra* by *C. asper* below the dam, reducing competition. Variable levels of competition should be based on differences in population density estimates in Catllar Stream, and this is indeed what the upstream/downstream population density differences showed: low *C. asper* density allows a higher density of *S. salamandra*.

On the other hand, trout are usually found in allopatry with *C. asper* on the southern slope of the Catalan Pyrenees, and in populations where they are sympatric, as in Catllar Stream, they show spatial and trophic segregation (Montori, 1988). Despite this, newts are usually a low-frequency component in the diet of trout, being eaten only occasionally. However, trout and newts do compete for the same trophic resources (Hartel et al., 2007; Montori and Llorente, 2014). Normal abundance estimates for this trout species in Pyrenean streams range from 0.36–0.90 individuals per m^2 (García de Jalón et al., 1986), which indicates that the trout population in Catllar Stream, with an estimated density of around 0.08 per m^2, is much lower than elsewhere. Trout subpopulations above and below the dam presented similar population densities and body sizes, which would indicate that they exert similar predation pressure on the respective *C. asper* subpopulations. Consequently, trout presence might be a factor that contributes to the overall low newt density in the stream, especially considering the fact that this stream has high water flow during autumn due to increased precipitation, and during spring and early summer due to the snowmelt, allowing trout to occupy otherwise shallow spots in the stream that could be suitable newt oviposition sites. Despite this, as a similar trout density was found both above and below the dam, the observed density and size differences between the subpopulations of *C. asper* must be driven by factors other than predation and competition, and the availability of trophic resources must be considered.

Food resources for *C. asper* in Catllar Stream. The diet of *C. asper* is primarily based on aquatic macroinvertebrates, mainly different groups of insect larvae, with < 10% of their diet based on terrestrial prey (Montori and Llorente, 2014). These newts have different dietary needs during different life stages. Whereas adults are generalists and feed on small-sized macroinvertebrates, larval newts focus on the larvae of true flies and mayflies (Montori, 1991; Montori and Llorente, 2014). As we observed in the previous environmental characterization

Figure 2. Deformed leg of an individual of the upstream subpopulation of *Calotriton asper*, showing a front leg composed exclusively of one finger. Photo by Alejandro López de Sancha.

of Catllar stream, mayflies, stoneflies, and caddisflies, as well as nonbiting midges (the main true fly group found in the stream), were highly abundant, and their biomass and biodiversity index did not differ above or below the dam. Consequently, *C. asper* did not lack food resources in its larval stage or as adults in this stream. This was also confirmed by the lack of differences in the physiological condition between stream subpopulations. It seems that prey availability was not a limiting factor for *C. asper* in this stream, and the low population densities and the differences between upstream and downstream subpopulations in this stream might instead be caused by abiotic factors.

Metal and metalloid pollution diminishes population density and body size of *C. asper*. After eliminating biotic factors as causes for the differences in densities observed in *C. asper* subpopulations, one remaining feasible explanation is the presence of metal and metalloid pollution in the stream. Metal pollution is considered one of the major drivers of worldwide amphibian population declines (e.g., Blaustein et al., 2003). Amphibians are useful bioindicators of pollution due to their toxin absorption through respiration and sediment ingestion gulped during predation (Adlassnig et al., 2013; Dovick et al., 2020). In Catllar Stream, we documented a significantly higher concentration of arsenic in the water, and of arsenic, nickel, and copper in biofilms, upstream from the dam. Other metals, such as chromium, zinc, cadmium, and antimony, were also found throughout the stream. All of these metals and metalloids have been shown to cause negative effects on amphibians, including deformities, delayed development and reduced size, changes in antipredator behaviour, and reduction in fitness and survival (Nebeker et al., 1995; Chen et al., 2009; Gay et al., 2013; Adlassnig et al., 2013; Dovick et al., 2020).

Gardner et al. (2018) studied how environmental exposure to arsenic and chromium affected *Ambystoma gracile* (Baird, 1857), and these authors showed that water concentrations $\geq$ 5.99 µg/l for arsenic and $\geq$ 1.45 µg/l for chromium caused bioaccumulation of these metals in the salamanders' organs, altering their functioning and causing DNA damage. Moreover, both metals can be transferred from parents to offspring (Magari et al., 2002; Gardner et al., 2018). Levels of chromium in Catllar Stream were similar to the ones found in the *Ambystoma* study, and arsenic levels were much higher, especially upstream from the dam. *Calotriton asper* could be affected in the same way as *A. gracile*, considering the phylogenetic closeness of newts to ambystomatid salamanders and their primarily aquatic lifestyle (Montori, 1988), Thus, their exposure to pollution might be greater than experienced by the more terrestrial *A. gracile*. In our study, we found individuals with deformities in both subpopulations. Upstream newts were on average smaller than those downstream, and considering the deleterious effects of metals on amphibian size, development, and survival, and the lack of differences in food resources and predator pressure between the sites, metal pollution is one of the most likely causes for the difference in population density and body size of *C. asper* in Catllar Stream.

It is also possible that size differences between subpopulations could be caused by temperature differences on either side of the dam because the size of ectothermic species is dependent on water temperature: warmer water allows for an increase in metabolic activity and concomitant growth. However, it is known that *C. asper* populations at higher elevations and from colder water are larger than ones from lower elevations (Trochet et al., 2019) and in this stream, warmer water temperatures exist below the dam where the average size of individuals is larger. Predation pressure also affects body size (Diego-Rasilla, 2003), but trout density and size were similar at both sites. It could also be argued that the upstream subpopulation might be younger overall than the downstream one but given the longevity and sedentary lifestyle of this species and the presence of only adult individuals these are improbable explanations, leaving the metal pollution hypothesis far more feasible.

Damming impacts on newts in Catllar Stream. It is known that stream regulation has a profound impact on the hydrological regime and connectivity of streams, causing habitat fragmentation (Bednarek, 2001). Migration in *C. asper* is key for the colonization of new habitats to offset the passive downstream drift and to re-colonize habitats after floods (Montori et al., 2008), so the presence of a dam in Catllar Stream might be another cause for the low overall newt population density. This dam presents an impassable barrier for *C. asper*, for newts both in and out of the water, which may be leading to fitness loss due to inbreeding and even to local extinctions. This kind of threat is stronger for small populations, such as the one in Catllar Stream (Montori et al., 2007; Miró et al., 2018). Moreover, water diversion through the dam reduces the downstream water concentration of metals and metalloids and improves water quality there, likely causing the differences we observed between subpopulations: higher population density and body size of *C. asper* in the low-metal concentrations downstream from the dam.

Chytridiomycosis could become exacerbated in hydrologically altered Pyrenean streams. The Catllar Stream population of *C. asper* is so far unaffected by chytridiomycosis, but it is certainly susceptible. The most recent review on the prevalence of *Bd* and *Bsal* in the Pyrenees (Martínez-Silvestre et al., 2020) indicated that *Bsal* still had not been reported in any Pyrenean population of *C. asper*, and that *Bd* was found only in five Spanish populations among 29 populations sampled throughout the range. One of these affected populations is in Camprodon, only 8 km away from our studied population, making the Catllar Stream population susceptible to the infection. The susceptibility to this disease depends also on water temperature (Walker et al., 2010; Clare et al., 2016; Greenspan et al., 2017). Consequently, the predicted warming of Pyrenean waters due to climate change and the water flow reduction and temperature increase that damming causes could further increase the risk of chytridiomycosis in Pyrenean streams affected by damming.

Research and management of newts are crucial for their conservation. *Calotriton asper* is not only an amphibian flagship species for the Pyrenees but also a key structural component for the correct functioning of Pyrenean headwater streams. It has an important role in trophic top-down control, it is a food resource for higher trophic levels, and it represents a link between aquatic and terrestrial environments (Sánchez-Hernández, 2020). Assessing and managing the state of small populations, such as the one in Catllar Stream, might not only be relevant for the survival of the species but fundamental to the conservation of larger populations (Montori et al., 2007). Studying small populations should be promoted to not only increase the availability of population data and assess the actual conservation status of this species but also to understand how anthropogenic threats found in many streams in the range of *C. asper* may impact or impair its survival. Case studies like ours provide new information about the role of *C. asper* as a bioindicator in a realistic and applied context, indicating how this species can be affected by current and rising threats like metal pollution and damming, providing relevant and applied knowledge to propose management actions that could be effective for the conservation of the headwater streams on which *C. asper* depends.

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